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Interferon-signaling pathway: associations with colon and rectal cancer risk and subsequent survival

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Interferons (IFNs) are proteins involved in many functions including antiviral and antimicrobial response, apoptosis, cell cycle control and mediating other cytokines. IFN gamma (IFNG) is a proinflammatory cytokine that modulates many immune-related genes. In this study we examine genetic variation in *IFNG*, *IFNGR*1, *IFNGR2* and interferon regulatory factors (IRFs) to determine associations with colon and rectal cancer and survival after diagnosis. We include data from two population-based incident studies of colon cancer (1555 cases and 1956 controls) and rectal cancer (754 cases and 959 controls). Five tagSNPs in *IFNG*, *IRF2* and *IRF3* were associated with colon cancer and eight tagSNPs in *IFNGR1*, *IFNGR2*, *IRF2*, *IRF4*, *IRF6* and *IRF8* were associated with rectal cancer. *IRF3* rs2304204 was associated with the strongest direct association and *IRF2* 3775554 with the strongest inverse association for colon cancer [odds ratios (ORs) 1.43, 95% confidence interval (CI) 1.12–1.82 for recessive model and 0.52, 95% CI 0.28–0.97 for unrestricted model]. For rectal cancer, *IFNGR1* rs3799488 was directly associated with risk (OR 2.30, 95% CI 1.04–5.09 for recessive model), whereas *IRF6* rs861020 was inversely associated with risk (OR 0.57, 95% CI 0.34–0.95). Several single-nucleotide polymorphisms interacted significant with both *NF-κB1* and *IL6* and with aspirin/non-steroidal anti-inflammatory drugs and cigarette smoking. Using a summary score to estimate mutational load, we observed a hazard rate ratio (HRR) close to 5.00 (95% CI 2.73–8.99) for both colon and rectal (HRR 4.83, 95% CI 2.34–10.05) cancer for those in the category having the most at-risk genotypes. These data suggest the importance of IFN-signaling pathway on colon and rectal cancer risk and survival after diagnosis.

Introduction

Interferons (IFNs) are proteins involved in many functions including antiviral and antimicrobial response, apoptosis, control of cell cycle and mediators of other cytokines (1,2). There are three classes of IFNs, type I, II and III. Interferon gamma (IFNG) is the only type II IFN and as a proinflammatory cytokine, has been identified as an important modulator of immune-related genes, including nuclear factor-kappa B (NF-κB), toll-like receptor 3 (TLR3), VCAM1 and CASP4 (3), interferon gamma receptor (IFNGR), interferon regulatory factors (IRF), V-AKT murine thymoma viral oncogene homolog 1 (AKT), mitogen-activated protein kinases and inhibitor of kappa (IKK) (1, 4). IFN receptors are required for IFNs to exert their biological activity and therefore play a critical role in IFN signaling (4,5); IFNGRs have two subunits, IFNGR1 and IFNGR2. IRFs are a family of transcription factors (2,6) involved in the regulation of the IFN system, cell growth and the regulation of host defense such as innate and adaptive immune response.

Abbreviations: CI, confidence interval; HRR, hazard rate ratio; IFN, interferon; IFNG, interferon gamma; IFNGR, interferon gamma receptor; IRF, interferon regulatory factor; KPMCP, Kaiser Permanente Medical Care Program; NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio.

The IFN-signaling system may play a critical role in carcinogenic processes. However, few studies of genetic variation in the IFN-signaling pathway have been examined with colon or rectal cancer. Of these genes, only IFNG has been examined, perhaps because of its role in maintaining the integrity of the intestinal epithelial barrier (7). *IFNG* -874T > A (rs2430561) was not associated with risk of hereditary non-polyposis colon cancer in a study of 212 cases (8). A small study of 170 colon and rectal cancer cases in Korea did not find an association with *IFNG* 5644 (9). Studies examining genetic variation in other components of the IFNG-signaling pathway have not been reported nor have studies examined the impact of genetic variation in this pathway on survival. Given the role of IFNG in apoptosis, cell growth and regulation, such an association is biologically plausible.

In this study, we examine the genetic variation in *IFNG*, *IFNGR1*, *IFNGR2*, *IRF1*, *IRF2*, *IRF3*, *IRF4*, *IRF5*, *IRF6*, *IRF7*, *IRF8* and *IRF9* with risk of developing colon and rectal cancer as well as their association with survival. Given the biological function of this signaling pathway, we evaluate interaction with two key inflammation-related genes, *NF-κB1* and *IL6* (10) as well as two lifestyle factors that may modify genetic susceptibility, use of aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs) and cigarette smoking. Both aspirin/NSAID use and cigarette smoking may modify associations through their influence on inflammation. Aspirin/NSAID use may reduce inflammation, whereas cigarette smoking may increase inflammation as a result of oxidative stress.

Methods

Two study populations are included. The first, a population-based case–control study of colon cancer, included cases (n = 1555) and controls (n = 1956) identified between 1 October 1991 and 30 September 1994 living in the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program (KPMCP) of Northern California and a seven-county area of Utah (11). The second study used identical data collection methods as the first study but included population-based cases with cancer of the rectosigmoid junction or rectum (n = 754) and controls (n = 959) who were identified between May 1997 and May 2001 in Utah and KPMCP (12). Eligible cases were between 30 and 79 years old at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of CRC and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis or Crohn's disease. Controls were matched to cases by sex and by 5 years age groups. At KPMCP, controls were randomly selected from membership lists. In Utah, controls ≥65 years were randomly selected from the Health Care Financing Administration lists and controls <65 years were randomly selected from driver's license lists. While in Minnesota, controls were selected from driver's license and state-identification lists. Study details have been previously reported (11,12).

Interview data collection

Data were collected by trained and certified interviewers using laptop computers. All interviews were audiotaped and reviewed for quality control purposes (13). The referent period for the study was 2 years prior to diagnosis for cases and prior to selection for controls. Detailed information was collected on diet, physical activity, medical history and cigarette smoking history, regular use of aspirin and NSAIDs and body size. Regular use of aspirin/NSAIDs was defined as at least three times a week for at least 1 month.

Tumor registry data

Tumor registry data were obtained to determine disease stage at diagnosis and months of survival after diagnosis. Disease stage was categorized centrally by one pathologist in Utah using the sixth edition of the American Joint Committee on Cancer (AJCC) staging criteria. Local tumor registries also provided information on patient follow-up including vital status, cause of death and contributing cause of death. Follow-up was obtained for all study participants for at least 5 years and was terminated for the Colon Cancer Study in 2000 and

for the Rectal Cancer Study in 2007. We used the standard definition of colon and rectal cancer employeed by the Surveillance and Epidemiology and End Results (SEER) program.

TagSNP selection and genotyping

TagSNPs were selected using the following parameters: linkage disequilibrium blocks were defined using a Caucasian linkage disequilibrium map and an $r^2 = 0.8$; minor allele frequency >0.1; range = −1500 bps from the initiation codon to +1500 bps from the termination codon and one single-nucleotide polymorphism (SNP)/linkage disequilibrium bin. All markers were genotyped using a multiplexed bead-array assay format based on Golden Gate chemistry (Illumina, San Diego, CA). A genotyping call rate of 99.85% was attained. Blinded internal replicates represented 4.4% of the sample set; the duplicate concordance rate was 100%. Individuals with missing genotype data were not included in the analysis for that specific marker. We evaluated associations with 12 candidate genes, including *IFNG* (3 SNPs), *IRNGR1* (4 SNPs), *IFNGR2* (5 SNPs), *IRF1* (2 SNPs), *IRF2* (51 SNPs), *IRF3* (2 SNPs), *IRF4* (10 SNPs), *IRF5* (4 SNPs), *IRF6* (5 SNPs), *IRF7* (2 SNPs), *IRF8* (12 SNPs) and *IRF9* (2 SNPs).

Tumor marker data

We have previously evaluated tumors for CpG island methylator phenotype, microsatellite instability, *TP53* mutations and *KRAS2* mutations (14–17) and were therefore able to evaluate genes in relation to tumors with specific characteristics or markers. Details for methods used to evaluate these epigenetic and genetic changes have been described in previous publications (14–17).

Statistical methods

Statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC). We report odds ratios (ORs) and 95% confidence intervals (95% CIs) assessed from multiple logistic regression models adjusting for age, center, race/ethnicity and sex. To summarize risk associated with multiple variants across the pathway, we created a summary polygenic score that was based on all at-risk genotypes for colon and rectal cancer. The score for each SNP was based on the inheritance model and its associated risk. For the codominant or additive model, a score of 0, 1 or 2 was assigned directly correlated to the number of high-risk alleles; scores of 0 or 2 were assigned for the dominant and recessive models. After assigning a score for each SNP previously identified as being significant, the scores were summed across SNPs to generate an individual polygenic summary score. Individuals missing SNP data were dropped from the analysis. The continuous score variable was redefined as a categorical variable based on the frequency distribution within the study population.

Analysis for interaction was based on tagSNPs within each gene. Lifestyle variables were selected because of their biological plausibility for involvement in this candidate pathway; in these analyses, we focused on interaction between cigarette smoking and use of aspirin/NSAIDs. We tested interaction with two genes, *NF-κB1* and *IL6*, which we hypothesized as importantly modifying the effect of candidate genes being analyzed given their importance in inflammatory processes. *P* values for interaction were determined using a likelihood-ratio test comparing a full model that included an ordinal interaction term with a reduced model without an interaction term.

Survival months were calculated based on month and year of diagnosis, and month and year of death or date of last contact. Associations between SNPs and risk of dying of colorectal cancer were evaluated using Cox proportional hazards models to obtain multivariate hazard rate ratios (HRRs) and 95% CIs. We adjusted for age at diagnosis, study center, race, sex, tumor molecular phenotype and AJCC stage to estimate HRRs.

Adjusted multiple comparison *P* values, taking into account tagSNPs within the gene, were estimated using the methods by Conneely *et al.* (18) via R version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). This method takes into account the correlated nature of the SNP data within a gene. Wald *P* values from the main effect models and interaction *P* values based on likelihood-ratio tests were used for estimates of multiple comparisons. We consider a pACT of <0.20 as being potentially important given the candidate pathway approach and the need to consider both type 1 and type 2 errors. We believe that findings at this level would merit replication, especially when evaluating interactions.

Results

The population characteristics are described in Table I. The colon cancer study consisted of cases and controls from all the three centers, whereas the rectal cancer study only included cases and controls for KPMCP and Utah. The majority of the population was non-Hispanic white, male and >60 years of age. The genes with corresponding tagSNPs that were associated with either colon or rectal cancer independently or through interaction with gene or lifestyle factors are described in Table II. All SNPs were in Hardy–Weinberg equilibrium. Roughly 90% of the population was non-Hispanic white. A summary of all SNPs analyzed can be found in the Supplementary Table, available at *Carcinogenesis* Online.

Table I. Description of study population

	Colon		Rectal	
	Control *n* (%)	Case *n* (%)	Control *n* (%)	Case *n* (%)
Total	1956	1555	959	754
Age				
30–39	40 (2.04)	23 (1.48)	21 (2.19)	19 (2.52)
40–49	128 (6.54)	102 (6.56)	101 (10.53)	96 (12.73)
50–59	326 (16.67)	290 (18.65)	243 (25.34)	196 (25.99)
60–69	673 (34.41)	538 (34.60)	329 (34.31)	250 (33.16)
70–79	789 (40.34)	602 (38.71)	265 (27.63)	193 (25.60)
Center				
Utah	378 (19.33)	249 (16.01)	365 (38.06)	274 (36.34)
KPMCP	787 (40.24)	744 (47.85)	594 (61.94)	480 (63.66)
Minnesota	791 (40.44)	562 (36.14)	0	0
Race/ethnicity				
NHW	1828 (93.46)	1428 (91.83)	824 (85.92)	625 (82.89)
Hispanics	75 (3.83)	59 (3.79)	63 (6.57)	61 (8.09)
Black	53 (2.71)	68 (4.37)	43 (4.48)	29 (3.85)
Asian	0	0	29 (3.02)	39 (5.17)
Sex				
Male	1047 (53.53)	870 (55.95)	541 (56.41)	451 (59.81)
Female	909 (46.47)	685 (44.05)	418 (43.59)	303 (40.19)
AJCC stage				
Stage I		469 (30.16)		381 (50.53)
Stage II		405 (26.05)		124 (16.45)
Stage III		374 (24.05)		175 (23.21)
Stage IV		128 (8.23)		57 (7.56)
Unknown		179 (11.51)		17 (2.25)
Tumor molecular phenotypes				
KRAS2 mutation		348 (22.38)		173 (22.94)
TP53 mutation		516 (33.18)		277 (36.74)
CIMP[a] high		272 (17.49)		59 (7.82)
MSI[a] unstable		185 (11.90)		14 (1.86)

[a]Tumor molecular phenotypes are CpG island methylator phenotype (CIMP) and microsatellite instability (MSI).

Five tagSNPs in three genes (*IFNG*, *IRF2* and *IRF3*) were associated with colon cancer (Table III) and eight tagSNPs in six genes (*IFNGR1*, *IFNGR2*, *IRF2*, *IRF4*, *IRF6* and *IRF8*) were associated with rectal cancer. The strongest increased risk was associated with *IRF3* rs2304204 for colon cancer (OR 1.43, 95% CI 1.12–1.82 for recessive model) and the strongest inverse association was observed for *IRF2* rs3775554 (OR 0.52, 95% CI 0.28–0.97 for unrestricted or codominant model or unrestricted). For rectal, rs3799488 of *IFNGR1* was associated with over a 2-fold increased risk (OR 2.30, 95 % CI 1.04–5.09 for recessive model), whereas *IRF6* rs861020 was associated with the strongest inverse association (OR 0.57, 95% CI 0.34–0.95). Only two SNPs in *IRF2* were associated with colorectal cancer when colon and rectal cancer were combined. The risk estimate for *IRF2* rs3733473 for colon cancer was 0.63 (95% CI 0.43–0.92), for rectal cancer was 0.97 (95% CI 0.61–1.53) and for the colorectal cancer was 0.74 (95% CI 0.55–0.99) with the association clearly being driven by colon cancer. On the other hand, a trend toward a protective effect of *IRF2* rs7655800 was seen for both colon and rectal cancer (OR 0.62, 95% CI 0.39–1.00 for colon cancer; OR 0.75, 95% CI 0.36–1.59 for rectal cancer; OR 0.66, 95% CI 0.44–0.98 for colorectal cancer). The Supplementary Table, available at *Carcinogenesis* Online, shows risk associated with all SNPs for colorectal cancer. Genes in this pathway appeared to be most uniquely associated with CpG island methylator phenotype + tumors (*P* for heterogeneity <0.05 for *IRF2* rs3733473, rs6812958 and *IRF6* rs17015218 for

Table II. Descriptive table of tagSNPs associated with colon and rectal cancer

Symbol	Chromosome location	Alias	SNP	Major/minor allele	MAF[a]	FDR HWE[b]
IFNG	12q14	IFG	rs1861493	A/G	0.31	1.00
		IFI	rs2069718	C/T	0.42	1.00
			rs2069727	A/G	0.46	1.00
IFNGR1	6q23–q24	CD119	rs1327474	A/G	0.46	1.00
		FLJ45734	rs1327475	C/T	0.16	1.00
		IFNGR	rs3799488	T/C	0.12	1.00
IFNGR2	21q22.11	AF-1	rs1532	C/T	0.30	0.95
		IFGR2	rs2834211	T/C	0.10	0.76
		IFNGT1	rs2834213	A/G	0.24	0.96
			rs2834215	G/A	0.45	0.87
IRF1	5q31.1	IRF-1	rs17622656	G/A	0.37	1.00
		MAR				
IRF2	4q34.1–q35.1	DKFZp686F0244	rs793801	G/A	0.40	0.22
		IRF2	rs809909	T/A	0.33	0.96
			rs965225	G/A	0.09	1.00
			rs2797507	C/A	0.46	1.00
			rs3733473	G/A	0.20	1.00
			rs3756093	C/G	0.15	0.61
			rs3756094	G/A	0.32	1.00
			rs3775554	G/C	0.13	1.00
			rs3775556	A/G	0.26	0.95
			rs3775574	A/G	0.40	0.92
			rs3822118	C/T	0.32	0.96
			rs6812958	G/A	0.28	0.79
			rs6827018	A/G	0.14	0.93
			rs6856910	T/C	0.33	1.00
			rs7655800	A/G	0.15	1.00
			rs9684244	G/C	0.37	1.00
			rs11132242	A/G	0.38	0.97
			rs12512614	G/T	0.24	0.68
			rs17488206	A/T	0.24	0.98
			rs17585389	T/C	0.27	1.00
IRF3	19q13.3–q13.4		rs2304204	A/G	0.26	0.95
IRF4	6p25–p23	LSIRF	rs872071	A/G	0.50	1.00
		MUM1	rs1050975	A/G	0.09	0.94
			rs11242865	C/T	0.20	0.94
			rs3778607	G/A	0.48	1.00
			rs3800262	G/A	0.17	1.00
			rs7768807	T/C	0.25	1.00
			rs12211228	G/C	0.14	1.00
IRF5	7q32		rs752637	G/A	0.37	0.89
			rs1874328	T/C	0.37	0.92
IRF6	1q32.3–q41	LPS	rs861020	G/A	0.21	1.00
		OFC6	rs2013162	C/A	0.38	1.00
		PIT	rs2013196	C/T	0.20	0.68
		PPS	rs17015218	A/G	0.16	0.97
		VWS				
IRF7	11p15.5	IRF-7H	rs1131665	A/G	0.26	1.00
		IRF7A				
IRF8	16q24.1	H-ICSBP	rs305084	T/C	0.09	0.97
		ICSBP	rs1044873	C/T	0.39	0.96
		ICSBP1	rs305071	G/A	0.12	0.96
		IRF8	rs13338943	G/T	0.11	1.00

[a]Minor allele frequency (MAF) based on control for non-Hispanic white population.
[b]FDR (HWE), false discovery rate adjusted *P* value for Hardy–Weinberg equilibrium test; HWE based on NHW control population (sample sizes range from 2453 to 2652).

colon cancer and *IFNG* rs2069718, *IRF2* rs2310047 and rs7657540 for rectal cancer) and *KRAS*-mutated tumors (*P* for heterogeneity <0.05 for *IRF6* rs2013162 for colon cancer and *IRF2* rs3775556 and *IRF8* rs8064189 for rectal cancer) (data not shown in table). Results were similar when analysis excluding non-Hispanic white individuals was performed.

We evaluate interactions between our candidate genes and *NF-κB1* and *IL6*, two genes we hypothesize as interacting with *IFN*-related genes given their role in inflammation. We have previously reported independent associations between *NF-κB1* and *IL6* and colon and rectal cancer (10,19) *IL6* rs2069860 was associated with reduced risk of colon cancer (adjusted OR 0.55, 95% CI 0.32–0.95). *NF-κB1* was associated with reduced risk of colon cancer (rs4648110 OR 0.66, 95% CI 0.45–0.96 for recessive model and rs13117745 OR 0.64, 95% CI 0.39–1.04 for recessive model). *NF-κB1* also was associated with rectal cancer (OR 0.79, 95% CI 0.51–0.94 for dominant model of rs23051; OR 1.32, 95% CI 1.00–1.75 for additive model of rs3821958; OR 1.24, 95% CI 1.03–1.51 for dominant model of rs11722146). We observe numerous interactions (Table IV). For colon cancer, *IFNG*, *IRF1*, *IRF2* interacted with *NF-κB1*, whereas *IFNGR1*, *IFNGR2*, *IRF2*, *IRF5*, *IRF6* and *IRF8* all interacted with *IL6*. For rectal cancer, we observed significant interactions between *NF-κB1* and *IFNG*, *IFNGR2*, *IRF4* and *IRF6*, whereas *IRFNGR1*, *IFNGR2*, *IRF1*, *IRF2* and *IRF8* interacted with *IL6*.

Table III. Associations between candidate SNPs and colon and rectal cancer

	Controls	Cases		Wald	
	N	*N*	OR[a] (95% CI)	*P* value	pACT
Colon					
IFNG (*rs1861493*)				0.0007	0.0019
AA	947	849	1.00		
AG/GG	1008	704	0.79 (0.69–0.91)		
IRF2 rs3775554 ($G > C$)				0.0205	0.5099
GG	1479	1218	1.00		
GC	443	322	0.88 (0.74–1.03)		
CC	34	15	0.52 (0.28–0.97)		
IRF2 (*rs793801*)				0.0112	0.3392
GG	666	590	1.00		
GA/AA	1290	964	0.84 (0.73–0.96)		
IRF2 (*rs809909*)				0.0097	0.3093
TT	878	626	1.00		
TA/AA	1078	929	1.20 (1.04–1.37)		
IRF3 (*rs2304204*)				0.0041	0.0080
AA/AG	1817	1397	1.00		
GG	139	157	1.43 (1.12–1.82)		
Summary score					
(0–2)	305	172	1.00		
(3–5)	911	653	1.27 (1.02–1.57)		
(6–7)	555	524	1.66 (1.33–2.08)		
(8–10)	185	206	1.92 (1.46–2.53)		
P_{trend}	<0.0001				
Rectal					
IFNGR1 (*rs3799488*)				0.0400	0.1120
TT/TC	949	737	1.00		
CC	10	17	2.30 (1.04–5.09)		
IFNGR2 (*rs2834211*)				0.0222	0.0949
TT	764	565	1.00		
TC/CC	195	189	1.31 (1.04–1.64)		
IRF2 (*rs3733473*)				0.0173	0.4518
GG	625	449	1.00		
GA/AA	334	305	1.27 (1.04–1.55)		
IRF2 (*rs3775556*)				0.0071	0.2338
AA	532	369	1		
AG	370	328	1.26 (1.03–1.54)		
GG	55	57	1.48 (0.99–2.19)		
IRF2 (*rs3775574*)				0.0029	0.1119
AA	317	297	1.00		
AG/GG	642	457	0.74 (0.60–0.90)		
IRF4 (*rs11242865*)				0.0184	0.1458
CC/CT	935	720	1.00		
TT	24	34	1.90 (1.11–3.24)		
IRF6 (*rs861020*)				0.0313	0.1296
GG/GA	910	732	1.00		
AA	49	22	0.57 (0.34–0.95)		
IRF8 (*rs1044873*)				0.0142	0.1317
CC/CT	789	654	1.00		
TT	170	100	0.71 (0.55–0.93)		
Summary score					
(0–4)	251	122	1.00		
(5–6)	390	280	1.47 (1.13–1.92)		
(7–8)	229	232	2.10 (1.58–2.79)		
(9–12)	89	120	2.79 (1.96–3.96)		

[a]OR and 95% CI adjusted for age, center, race/ethnicity and sex.

Significant interactions between recent regular use of aspirin/NSAIDs and smoking cigarettes with candidate genes are shown in Table V. Several genes interacted with aspirin/NSAIDs, including *IRF2*, *IRF4*, *IRF5* and *IRF6* for colon cancer and *IFNGR2*, *IRF2*, *IRF6* and *IRF7* for rectal cancer. Likewise, we observed several significant interactions between cigarette smoking and candidate genes. *IRF2* and *IRF4* interacted with smoking for both colon and rectal cancer. Additionally, there was significant interaction between *IRF6* and smoking for colon cancer and *IRF8* and smoking for rectal cancer.

We evaluate pathway tagSNPs with survival by looking at the mutational load using a summary score consisting of those SNPs associated with survival based on significant HRRs (Table VI). For colon cancer, the HRR was 4.96 (95% CI 2.73–8.99) for those in the category having the most at-risk genotypes; for rectal cancer, the upper summary HRR was 4.85 (95% CI 2.34–10.05) after adjusting for age, center, race, sex, AJCC stage and tumor molecular phenotype. Assessment of rectosigmoid junction separate from other rectal tumors showed similar results as for the combined group.

Discussion

Our data support the hypothesis that genetic variation in the *IFNG*, its receptors and *IRF* genes are associated with risk of developing colon and rectal cancer and that this association may be modified by other

Table IV. Associations between *IFNG*, *IRF* genes and *IL6*, *NFKB1* relative to wild-type/wild-type

IFNG, *IRF* gene	SNP (model)[a]	Pathway gene	SNP (model)	Wild-type[b] variant OR (95% CI)	Variant[c] wild-type OR (95% CI)	Variant[d] variant OR (95% CI)	Interaction *P* value	pACT
Colon								
IFNG	rs2069718 (A)	*NFKB1*	rs13117745 (D)	1.27 (0.98–1.63)	1.02 (0.81–1.29)	0.64 (0.46–0.89)	0.0015	0.0098
			rs4648090 (D)	1.12 (0.86–1.45)	0.95 (0.76–1.19)	0.63 (0.44–0.90)	0.0247	0.1174
			rs4648110 (D)	1.26 (1.00–1.60)	1.08 (0.85–1.37)	0.68 (0.50–0.93)	0.0008	0.0056
	rs2069727 (A)	*NFKB1*	rs11722146 (A)	1.28 (0.85–1.94)	1.44 (1.10–1.88)	1.19 (0.73–1.95)	0.0367	0.1611
			rs4648110 (D)	0.75 (0.59–0.97)	0.91 (0.72–1.15)	1.32 (0.99–1.75)	0.0014	0.0094
			rs4648127 (D)	0.83 (0.56–1.22)	1.07 (0.87–1.31)	1.63 (1.05–2.54)	0.0453	0.1877
IFNGR1	rs1327475 (D)	*IL6*	rs1800797 (A)	0.78 (0.62–0.99)	0.88 (0.69–1.12)	1.21 (0.86–1.71)	0.0142	0.1070
IFNGR2	rs2834213 (A)			0.98 (0.76–1.28)	1.55 (0.93–2.59)	0.36 (0.17–0.77)	0.0209	0.1851
IRF1	rs17622656 (A)	*NFKB1*	rs230510 (A)	0.86 (0.63–1.16)	0.64 (0.43–0.93)	1.00 (0.65–1.53)	0.0447	0.1451
IRF2	rs2797507 (A)	*IL6*	rs1800797 (A)	0.51 (0.35–0.76)	0.77 (0.57–1.04)	0.96 (0.62–1.47)	0.0039	0.2651
	rs3756094 (A)	*NFKB1*	rs4648090 (D)	1.19 (0.95–1.49)	1.39 (1.07–1.81)	0.74 (0.49–1.12)	0.0010	0.1115
	rs6812958 (A)	*IL6*	rs1800797 (A)	0.64 (0.48–0.85)	0.52 (0.34–0.81)	0.75 (0.39–1.43)	0.0014	0.1183
IRF5	rs752637 (A)		rs2069840 (A)	0.72 (0.51–1.01)	0.76 (0.55–1.04)	0.97 (0.57–1.64)	0.0227	0.1083
IRF6	rs17015218 (D)			0.75 (0.58–0.98)	0.83 (0.66–1.03)	1.05 (0.73–1.51)	0.0200	0.1568
IRF8	rs305071 (D)		rs2069827 (D)	0.75 (0.61–0.92)	0.94 (0.79–1.11)	1.20 (0.86–1.67)	0.0106	0.1762
Rectal								
IFNG	rs1861493 (A)	*NFKB1*	rs4648127 (D)	0.78 (0.49–1.24)	1.15 (0.79–1.67)	3.33 (1.03–10.74)	0.0097	0.0636
IFNGR1	rs1327474 (A)	*IL6*	rs1800796 (D)	1.83 (1.21–2.79)	0.94 (0.69–1.28)	0.76 (0.39–1.48)	0.0338	0.2140
IFNGR2	rs1532 (A)	*NFKB1*		1.87 (1.29–2.71)	1.25 (0.85–1.84)	0.35 (0.11–1.06)	0.0006	0.0069
			rs230510 (A)	0.91 (0.61–1.36)	1.66 (0.87–3.16)	1.06 (0.46–2.46)	0.0349	0.2953
	rs2834215 (A)	*IL6*	rs1800796 (D)	0.86 (0.50–1.48)	0.90 (0.66–1.22)	2.31 (1.38–3.85)	0.0026	0.0267
			rs2069840 (A)	1.17 (0.64–2.11)	1.79 (1.17–2.72)	1.41 (0.76–2.63)	0.0337	0.2466
IRF1	rs17622656 (A)		rs1800797 (A)	0.58 (0.35–0.96)	0.96 (0.56–1.66)	1.31 (0.65–2.66)	0.0382	0.1131
IRF2	rs12512614 (A)	*IL6*	rs1800796 (D)	0.98 (0.69–1.40)	1.01 (0.65–1.59)	4.38 (1.19–16.06)	0.0032	0.2299
	rs17585389 (A)		rs2069840 (A)	0.61 (0.39–0.94)	0.56 (0.29–1.08)	0.86 (0.35–2.13)	0.0034	0.2375
	rs2797507 (A)		rs1800796 (D)	2.09 (1.28–3.41)	1.19 (0.88–1.59)	0.70 (0.37–1.33)	0.0021	0.1622
IRF4	rs11242865 (D)	*NFKB1*	rs230510 (A)	0.85 (0.59–1.21)	1.25 (0.89–1.76)	0.52 (0.32–0.85)	0.0142	0.2432
	rs3800262 (D)		rs4648110 (D)	1.31 (1.03–1.67)	1.43 (1.10–1.84)	1.01 (0.74–1.39)	0.0046	0.0994
	rs7768807 (A)		rs4648110 (D)	1.33 (1.02–1.73)	1.66 (1.02–2.70)	1.06 (0.62–1.81)	0.0132	0.2314
IRF6	rs2013196 (D)		rs230510 (A)	0.89 (0.62–1.27)	1.47 (1.03–2.08)	0.59 (0.37–0.93)	0.0146	0.1451
			rs4648090 (D)	0.86 (0.65–1.12)	0.98 (0.77–1.24)	1.62 (1.13–2.33)	0.0056	0.0661
			rs4648110 (D)	0.89 (0.70–1.13)	0.93 (0.72–1.20)	1.52 (1.10–2.10)	0.0053	0.0621
IRF8	rs13338943 (D)	*IL6*	rs1800796 (D)	1.54 (1.14–2.08)	1.07 (0.83–1.38)	0.67 (0.36–1.25)	0.0107	0.1885

[a]Models: A, additive or codominant; D, dominant.
[b]Compares wild-type (WT) IFNG/IRF gene and variant from additive model or heterozygote/variant if dominant model for pathway SNP relative to both WT.
[c]Compares variant from additive model or heterozygote/variant if dominant model for IFGN/IRF gene and WT pathway gene relative to both WT.
[d]Compares variant from additive model or heterozygote/variant if dominant model for both IFNG/IRF and pathway gene relative to both WT.

key inflammation-related genes and lifestyle factors such as use of aspirin/NSAID and cigarette smoking. Additionally, we provide support for the hypothesis that genetic variation in the IFNG-signaling pathway is associated with survival. The increased risk of both developing colon or rectal cancer and survival after diagnosis appears to be influenced by mutational load. Our data suggest that unique associations were observed for CpG island methylator phenotype + and *KRAS2*-mutated tumors, suggesting that these tumor molecular phenotypes may be associated with inflammation.

IRF2 was associated with both colon and rectal cancer, whereas other components of the pathway were uniquely associated with colon cancer, (i.e. *IFNG* and *IRF3*), and with rectal cancer (i.e. *IFNGR1*, *IFNGR2*, *IRF4*, *IRF6* and *IRF8*). Although we acknowledge that these differences could stem from chance findings, many associations remained significant after adjusting for multiple comparisons. These findings also could support other reports showing differences in both genetic and lifestyle factors for colon and rectal cancer (12,20–23). For instance, body size and insulin signaling may play a larger role in the etiology of colon versus rectal cancer (21,23,24). Studies have shown that IFNG attenuates insulin signaling (25); thus an association between IFNG and colon cancer may reflect different biological components of colon versus rectal cancer.

Inflammation is a key element in colon and rectal carcinogenesis. We evaluated the interaction of IFN-signaling pathway genes with *NF-κB1* and *IL6*, two genes that appear to be pivotal in inflammatory response. All genes, except *IRF3*, *IRF4* and *IRF9* for colon cancer, and *IRF3*, *IRF5*, *IRF7* and *IRF9* for rectal cancer showed significant interaction with these genes. Others have shown that NF-κB1 expression is influenced by the IFN-signaling pathway (3). We interpret these findings to indicate both the importance of IFN-signaling pathway to an inflammation-related mechanism as well as the degree to which multiple inflammation factors work together to influence cancer risk. Although beyond the scope of this paper, we believe that it is important to examine how IFN genes work with other inflammation-related genes given the number of interactions observed. Genes that may be important include tumor necrosis factor and its receptors, toll-like receptors, mitogen-activated protein kinases including p38, mitogen-activated protein kinase 8 and mitogen-activated protein 14, inhibitor of kappa light chain gene enhancer in B cells, kinase of Beta (IKKB), cytokines such as interleukin 1 and interleukin 8 and AKT in addition to angiogenesis genes such as vascular endothelial growth factor and its receptors.

Our data suggest that genetic susceptibility is influenced by regular use of aspirin/NSAID use and smoking cigarettes. The role of aspirin and NSAID use in colon and rectal cancer risk are well documented (26–29). These associations are felt to stem from the anti-inflammatory properties of these drugs. Cigarette smoking has been associated with increased nitric oxide (NO) synthesis by activating nitric oxide synthase (NOS2) (30,31); NO has been shown to contribute to chronic inflammation (32). While multiple genes were associated with both colon and rectal cancer, *IRF2* and *IRF6* were associated with aspirin/NSAID use for both colon and rectal cancer and *IRF2* was associated with cigarette smoking for both colon and rectal cancer. Few studies have examined how either aspirin/NSAID or cigarette smoking works with these genes, although the interaction with genes in the IFN-signaling pathway is biologically plausible. One study has shown that

Table V. Interaction between cigarette smoking, NSAID use and *IFNGR2*, *IRF* genes and risk of colon and rectal cancer relative to wild-type/wild-type

IFNG, IRF genes	SNP (model)[a]	Variant[b]		Wild-type	Interaction *P* value	pACT
		No regular aspirin/NSAID use OR (95% CI)	Regular aspirin/NSAID use OR (95% CI)	Regular aspirin/NSAID use OR (95% CI)		
Colon						
IRF2	rs3756093 (D)	1.17 (0.97–1.41)	0.56 (0.44–0.71)	0.74 (0.62–0.87)	0.0070	0.2194
	rs3822118 (A)	0.84 (0.62–1.13)	0.84 (0.56–1.24)	0.58 (0.47–0.71)	0.0349	0.6535
	rs6856910 (A)	1.24 (0.94–1.65)	0.52 (0.36–0.76)	0.79 (0.64–0.98)	0.0047	0.1631
	rs9684244 (A)	1.23 (0.94–1.60)	0.65 (0.46–0.90)	0.85 (0.68–1.07)	0.0056	0.1871
IRF4	rs1050975 (D)	0.83 (0.67–1.03)	0.76 (0.58–1.01)	0.60 (0.51–0.70)	0.0189	0.1411
	rs3778607 (A)	0.90 (0.71–1.14)	0.73 (0.56–0.97)	0.54 (0.41–0.72)	0.0454.	0.2766
IRF5	rs1874328 (A)	1.01 (0.77–1.31)	0.77 (0.54–1.10)	0.53 (0.42–0.67)	0.0338	0.0960
IRF6	rs2013162 (A)	0.72 (0.56–0.94)	0.64 (0.46–0.88)	0.53 (0.42–0.67)	0.0136	0.0600
	rs2013196 (D)	0.80 (0.67–0.96)	0.64 (0.52–0.80)	0.59 (0.49–0.70)	0.0410	0.1525
Rectal						
IFNGR2	rs1532 (A)	0.75 (0.47–1.20)	0.82 (0.47–1.44)	0.54 (0.41–0.71)	0.0094	0.0438
IRF2	rs3756093 (D)	1.08 (0.82–1.43)	0.54 (0.38–0.76)	0.79 (0.63–1.00)	0.0415	0.7038
	rs3775574 (D)	0.87 (0.67–1.14)	0.51 (0.38–0.68)	0.92 (0.66–1.26)	0.0303	0.6056
	rs3822118 (A)	0.63 (0.41–0.98)	1.18 (0.69–2.00)	0.51 (0.38–0.69)	0.0004	0.0162
	rs965225 (D)	1.11 (0.80–1.55)	0.48 (0.32–0.74)	0.76 (0.61–0.94)	0.0451	0.7242
IRF6	rs861020 (D)	1.17 (0.90–1.52)	0.61 (0.45–0.83)	0.82 (0.64–1.05)	0.0289	0.1234
IRF7	rs1131665 (A)	1.91 (1.15–3.16)	0.55 (0.31–0.97)	0.82 (0.63–1.07)	0.0141	0.0280
		Non-recent smoker	Recent smoker			
Colon						
IRF2	rs6827018 (D)	1.12 (0.95–1.32)	0.97 (0.71–1.33)	1.29 (1.06–1.57)	0.0442	0.7598
IRF4	rs11242865 (D)	0.92 (0.79–1.08)	1.51 (1.15–1.98)	0.96 (0.77–1.19)	0.0030	0.0239
	rs12211228 (D)	0.90 (0.75–1.06)	1.59 (1.17–2.18)	1.00 (0.82–1.22)	0.0035	0.0256
	rs872071 (A)	1.21 (0.98–1.50)	1.04 (0.73–1.48)	1.93 (1.39–2.68)	0.0007	0.0062
IRF6	rs861020 (D)	1.13 (0.97–1.32)	1.02 (0.78–1.34)	1.35 (1.09–1.68)	0.0237	0.1011
Rectal						
IRF2	rs11132242 (A)	1.33 (0.96–1.84)	0.7 (0.40–1.48)	1.69 (1.14–2.49)	0.0118	0.3415
	rs12512614 (A)	1.42 (0.91–2.21)	1.01 (0.35–2.95)	1.64 (1.18–2.28)	0.0214	0.5131
	rs17488206 (D)	0.83 (0.67–1.03)	1.69 (1.13–2.52)	0.97 (0.70–1.34)	0.0053	0.1862
	rs3756094 (A)	1.14 (0.78–1.66)	0.53 (0.24–1.18)	1.56 (1.08–2.28)	0.0272	0.5866
	rs7655800 (D)	0.98 (0.78–1.25)	1.94 (1.22–3.06)	1.10 (0.81–1.48)	0.0428	0.7340
IRF4	rs3800262 (D)	1.27 (1.02–1.59)	1.09 (0.71–1.67)	1.57 (1.16–2.13)	0.0269	0.1848
	rs7768807 (A)	1.56 (1.05–2.31)	0.55 (0.21–1.44)	1.61 (1.16–2.24)	0.0063	0.0530
IRF8	rs305084 (D)	0.83 (0.62–1.11)	2.26 (1.18–4.32)	1.14 (0.87–1.50)	0.0198	0.1654

[a]Models: A, additive or codominant; D, dominant.
[b]Heterozygote/variant genotype if dominant model, variant if recessive; all comparisons are made to non-user/smoker and wild-type.

the NOS2 promoter contained sequences for several transcription factors including IRF6; exposure to tobacco smoke caused IRF6 to bind to the NOS2 promoter regulating NOS2 transcription and the cell response to tobacco exposure (31). Other studies like the one by Ratovitski will provide additional insight into the functionality of these genes.

Functions of the IFN-signaling pathway include apoptosis and cell proliferation. IRF1 has been shown to play a role in suppression of growth of breast cancer cells (33). IFNG has been shown to regulate the expression of apoptosis-related genes and has been hypothesized to regulate cell sensitivity to apoptosis (34). Additionally, IFNG has been shown to work with tumor necrosis factor to overcome resistance of metastatic colon tumor cells to the tumor necrosis factor-related apoptosis-inducing ligand, which is an immune effector molecule (35). Our observations that genetic variations in the IFN-signaling pathway influence survival have merit. The observed risk associated with multiple variants within the pathway further suggests that the mutational load is important. With increasing number of variant genotypes, the risk of dying increased. Although one could hypothesize that a single insult to the pathway could influence risk and that additional insults would have minimal effect on risk, our data suggest otherwise. Inflammatory pathways are somewhat redundant, composed of multiple cytokines with overlapping functions; this supports that multiple insults to the pathways would result in increased risk. Our data support the hypothesis that increases in risk and hazard of dying is linear and that as mutational load of high-risk genotypes increase, so does the risk of developing cancer and dying after being diagnosed with cancer. Our observed increased risk of dying was independent of disease stage at diagnosis and tumor molecular phenotype.

Major strengths of our study were the hypothesis-driven approach, the large and extensive data set includes information on genetic, lifestyle, tumor and survival data, and our ability to examine colon and rectal cancer separately. Although we believe that the data we present is both thorough and informative, we acknowledge that limitations exist. For instance, while we have detected associations, we have minimal information on the functionality of SNPs evaluated. Additional lab-based work is needed to determine functionality. We have limited our assessment of interaction to *NF-κB* and *IL6*, although other genes such as *TLR3*, *VCAM3* and *CASP4* were not considered. Additionally, we have made many comparisons. We have provided pACT values, which account for these comparisons although chance findings may exist. A hazard of multiple testing adjustments is the increased likelihood of rejecting a finding that is true. Thus, we believe that adjusted *P* values of <0.20, especially for interactions, merit replication in other large sample sets to validate these findings. Our assessment was limited to those enrolled in the study; those with the poorest survival were less likely to be included which, however, we did not observed differences in association when we examined disease stage at time of diagnosis.

We conclude that genetic variation in the IFN-signaling pathway is important in the etiology of colon and rectal cancer. These associations appear to be modified by lifestyle factors such as aspirin/NSAID use and cigarette smoking and other inflammation-related genes. Additionally, our data suggest the importance of genetic variation in this pathway on survival after diagnosis. We encourage validation of these findings in other large studies.

Table VI. Association between survival and IFN-related genes adjusted for age, center, race, sex, AJCC stage and tumor molecular phenotype

	Death/person-years	HRR (95% CI)	Wald test *P* value	FDR
Colon				
IFNGR1 (rs1327474)			0.0202	0.0405
AA/AG	261/6417	1.00		
GG	48/1731	0.69 (0.50–0.94)		
IFNGR1 (rs9376267)			0.0073	0.0291
CC	181/5109	1.00		
CT/TT	128/3039	1.37 (1.09–1.73)		
IFNGR2 (rs2834211)			0.0396	0.1979
TT	233/6468	1.00		
TC/CC	76/1680	1.32 (1.01–1.72)		
IRF2 (rs12504466)			0.0037	0.0801
TT	67/2349	1.00		
TC/CC	242/5799	1.51 (1.14–1.99)		
IRF2 (rs13116389)			0.0065	0.0801
GG	190/5662	1.00		
GT/TT	119/2486	1.38 (1.09–1.75)		
IRF2 (rs2797507)			0.0356	0.2597
CC	107/2528	1.00		
CA/AA	202/5620	0.77 (0.61–0.98)		
IRF2 (rs3775582)			0.0053	0.0801
GG	248/6222	1.00		
GA/AA	61/1926	0.67 (0.50–0.89)		
IRF2 (rs7655800)			0.0210	0.1787
AA	212/6142	1.00		
AG/GG	97/2006	1.33 (1.04–1.70)		
IRF2 (rs793777)			0.1174	0.4554
CC	134/3058	1.00		
CG	141/3829	0.89 (0.70–1.14)		
GG	34/1260	0.67 (0.46–0.98)		
IRF2 (rs793801)			0.0429	0.2732
GG/GA	262/6911	1.00		
AA	47/1232	1.39 (1.01–1.91)		
IRF2 (rs793814)			0.0036	0.0801
TT/TA	277/7120	1.00		
AA	32/1015	0.57 (0.39–0.83)		
IRF2 (rs9684244)			0.0079	0.0801
GG/GC	282/6923	1.00		
CC	27/1225	0.58 (0.39–0.87)		
IRF6 (rs2013196)			0.0368	0.1840
CC	190/5451	1.00		
CT/TT	119/2681	1.29 (1.02–1.63)		
IRF8 (rs1044873)			0.0251	0.1503
CC	101/3073	1.00		
CT/TT	208/5075	1.32 (1.04–1.68)		
IRF8 (rs305083)			0.0218	0.1503
AA	184/5181	1.00		
AG/GG	125/2967	1.31 (1.04–1.65)		
Summary score				
(2–10)	26/1204	1.00		
(11–12)	32/1018	2.06 (1.21–3.49)		
(13–14)	45/1440	2.13 (1.31–3.48)		
(15–16)	55/1465	2.03 (1.27–3.26)		
(17–18)	50/1381	3.00 (1.85–4.87)		
(19–20)	46/851	3.43 (2.10–5.60)		
(21–22)	35/497	4.26 (2.53–7.19)		
(23–28)	20/293	4.96 (2.73–8.99)		
P_{trend}		<0.0001		
Rectal				
IFNGR2 (rs2834213)			0.0130	0.0652
AA/AG	155/4084	1.00		
GG	16/205	2.04 (1.16–3.57)		
IRF2 (rs1425551)			0.0366	0.4670
AA/AC	133/3334	1.00		
CC	38/956	1.50 (1.03–2.18)		
IRF2 (rs3756094)			0.0009	0.0481
GG/GA	158/3866	1.00		
AA	13/423	0.36 (0.20–0.66)		
IRF2 (rs3822118)			0.0157	0.2669
CC	73/2047	1.00		
CT/TT	98/2242	1.47 (1.08–2.01)		

Table VI. *Continued*

	Death/person-years	HRR (95% CI)	Wald test *P* value	FDR
IRF2 (rs807684)			0.0025	0.0639
AA/AG	164/3944	1.00		
GG	7/346	0.30 (0.14–0.66)		
Summary score				
(0–2)	11/558	1.00		
(4–4)	41/984	2.68 (1.36–5.31)		
(6–6)	94/2227	3.32 (1.75–6.29)		
(8–10)	25/521	4.85 (2.34–10.05)		
P_{trend}		<0.0001		

Supplementary material

The Supplementary Table can be found at http://carcin.oxfordjournals.org/

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